

Larval feeding behaviour and myrmecophily of the Brenton Blue, *Orachrysops niobe* (Trimen) (Lepidoptera: Lycaenidae)

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Abstract. The larval feeding behaviour and myrmecophily of the Brenton Blue *Orachrysops niobe*, an endangered polyommata butterfly from Knysna in South Africa, were investigated by field observations and captive larval rearing. The aerial and subterranean parts of the *Indigofera erecta* legume host plants were searched for *O. niobe* eggs, larvae and potential host ants. Third and fourth instar larvae and pupae were found in association with *Camponotus baynei* ants on the host plant rootstock. Ant colonies in viewable artificial *C. baynei* nests were sited near host plants bearing multiple *O. niobe* eggs, but no larvae were taken into the nests. Cannibalism was observed between larvae raised in captivity on cut host plant. A third instar captive larva was enclosed with a potted host plant connected to a similar artificial ant nest. The larva disappeared and was later found feeding on the depleted plant rootstock, tended by the ants, and this behaviour was confirmed by field observations. *O. niobe*'s ant association is inferred to be obligate. Leguminous *Indigofera* host plants have amino acid enriched rootstocks, which may have pre-adapted the larval digestive system to a cannibalistic or carnivorous lifestyle. Larval growth characteristics are used to compare African polyommata genera and *Orachrysops* is intermediate between the facultative myrmecophilous genera and the predaceous/parasitic *Lepidochrysops* species. A cladistic analysis based on host plants, ant associations and feeding behaviour leads to a hypothetical phylogeny of the African myrmecophilous polyommata.

Key words: myrmecophily, *Orachrysops niobe*, polyommata, phytophagy, rootstock feeding.

INTRODUCTION

South Africa has a wealth of myrmecophilous lycaenids (Terblanche & van Hamburg, 2003), many of which exhibit restricted ranges (endemism) and are Red Listed species (Henning & Henning, 1989; Henning *et al.*, 2009). The phenomena of endemism and rarity are believed to result from the narrow environmental niches available to species that require the overlapping presence of host plants and tending ants (Pierce *et al.*, 2002). Nearly all of the obligately myrmecophilous South African lycaenid butterflies are in the tribes Aphnaeini and Polyommataini (*sensu* Pringle *et al.*, 1994).

Orachrysops is a recently erected polyommata genus (Vári & Kroon, 1986), for which the life history and myrmecophily of its 11 species and one subspecies are little known. Clark and Dickson (1971) were only able to rear larvae of *Orachrysops lacrimosa* (Bethune-Baker, 1923) to the end of the second instar, after which the larvae died. Recent work on the two most

endangered species in the genus has extended this knowledge to all stages of their life history. Edge and Pringle (1996) reported that the larvae of *Orachrysops niobe* (Trimen, 1862) were phytophagous in all instars during captive rearing to the adult stage, and whilst a dorsal nectary organ (DNO) was present no ant association appeared to be necessary. Lu and Samways (2001; 2002a; 2002b) made field observations of all larval stages and pupae for *Orachrysops ariadne* (Butler, 1898) and detected an apparently obligate ant association with *Camponotus natalensis* (F. Smith).

Polyommata larvae display a range of ant associations, including predaceous parasitism, facultative mutualisms and myrmecoxeny (no ant association). Larval diets vary from phytophagy to entomophagy, or combinations thereof (Cottrell, 1984; Fiedler, 1991b; Fiedler, 1998; Pierce *et al.*, 2002). Variation is evident within genera (e.g. *Maculinea*) as well as between genera, with significant implications for the ecology and population dynamics of each species (Thomas *et al.*, 1998). Consequently extrapolations between even closely related species can be misleading, and detailed field observations as well as laboratory experiments are the only way to establish with any certainty the larval diet and the exact nature of the myrmecophily for each species (Thomas *et al.*, 1989; Elmes & Thomas, 1992).

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MATERIALS AND METHODS

Study site

The study site was the Brenton Blue Butterfly Reserve (BBBR) at Brenton-on-Sea near Knysna in the Western Cape Province of South Africa. It has a total area of 14,670 m², is centred at co-ordinates 34°04'20" S, 23°02'00" E, and lies at 90–115 metres above mean sea level on a well-drained south-facing slope with an average inclination of 1 in 3 (18°), varying between 10° and 26°. The climatic, topographical and geological features of the site and its vegetation communities have been fully described elsewhere (Edge, 2005; Edge *et al.*, 2008a).

Field observations

All *O. niobe* host plants (*Indigofera erecta* Thunberg, Fabaceae) were systematically searched for eggs between November 2001 and April 2003 and all plants with >5 eggs were searched repeatedly to detect the presence of any larvae, pupae or ants, at various times of day including the evening. The size, stage and behaviour of any larvae discovered were recorded, and samples were taken of ants for identification. Sizes were measured with a vernier scale using a hand lens. From April 2002 not only were the leaves and stems of the plants down to ground level searched, but also some of the rootstocks were carefully excavated to a depth of 2–4 cm.

Captive rearing on cut host plant

Host plant sprigs bearing eggs were cut off and placed in clear air-tight plastic containers 25 mm diameter x 55 mm high, with a drop of water maintained in the bottom of the container to prevent desiccation of the plant. The oviposition date (if known), hatching date and all subsequent dates and measurements were written on labels attached to the container lids. If there were two or more ova on a sprig, the larvae were separated into individual containers on emergence. Every few days the contents were carefully removed, the larvae examined and the overall length (from the tip of the mandibles to the end of the final segment for the first instar larvae, and of the dorsal carapace for the second, third and fourth instars) measured to an accuracy of 0.5 mm with a vernier calliper. The containers were cleaned out with water and fresh cut sprigs of host plant were inserted after carefully transferring the larvae to the new leaves. From the third instar onwards, the larvae were transferred to larger flatter plastic containers 90

mm diameter x 50 mm high that would accommodate larger pieces of host plant. Upon pupation, the pupae were removed and placed on cotton wool under a netting eclosion cage. Any adults emerging were preserved as voucher specimens.

Artificial ant nests

Artificial ant nests similar to those used by Britton (1997) 300 mm x 150 mm x 20 mm deep, with labyrinthine passages, were made from wood with sealed transparent tops, and covered by a detachable hardboard lid to exclude light. Three queen right colonies of the host ant *Camponotus baynei* Arnold were collected on 5 October 2002, at a location away from the BBBR, by breaking open decayed logs lying on the ground under dense bushes. Each ant colony was kept in a large plastic box 320 x 220 x 60 mm deep with fluon (active ingredient: polytetrafluoroethylene) coated walls to prevent escape, and the ants were offered access into one of the artificial nests through a translucent plastic tube. The ants quickly took up residence in the artificial nests and feeding stations were set up in the large plastic box where a 50% v/v solution of sugar, plain water and chopped up dead insects was provided. On several occasions a third instar larva of *O. niobe* on its sprig of host plant was placed in the plastic box to observe any ant interactions.

Two of these ant nests were slightly buried (covered with 10–20 mm soil) on 16 October 2002, close to host plants on which a large number of *O. niobe* eggs had been laid, to see whether butterfly larvae would be taken into the artificial ant nest. Translucent plastic tubing provided access from the ant nest to the base of the host plant. The nests were inspected every week until 27 January 2003, when one of the nests was removed to the laboratory to prepare for a captive rearing experiment (see below). The other nest remained in the field until January 2004, when it too was removed to the laboratory for another captive rearing experiment.

Captive rearing with live host plant and ants

Two *I. erecta* plants were transplanted from the field with their surrounding soil into pots 175 mm diameter x 100 mm deep in December 2002, and watered regularly. An experiment was set up in February 2003 with the two potted and caged *I. erecta* plants, an artificial ant nest containing an ant colony with brood of all stages, and one of the large plastic boxes with ant feeding stations, all connected by clear 6 mm diameter plastic tubes (Fig. 1). A 3rd instar larva

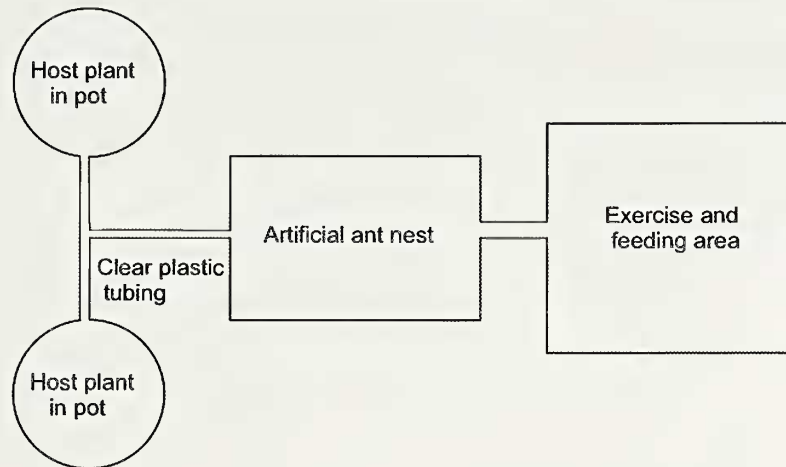


Figure 1. Experimental set up for captive rearing with live host plant and ants

(7 mm long) was placed on each of the *I. erecta* plants on 8 March 2003. The plants and the ant nest were examined regularly to detect any larval activity and any ant-larva interactions.

Morphology of the immature stages of *O. niobe*

Larvae were examined with a Wild M5 stereomicroscope at magnifications of up to 50x. The various stages were photographed under magnification with a Nikon Coolpix E4600 digital camera. Particular attention was given to the dorsal nectary organs (DNOs), perforated cupola organs (PCOs), tentacular organs (TOs) and the mandibles of the 4th instar larva.

Growth characteristics of *O. niobe* larvae and comparison with other polyommatae

Data were obtained from Clark and Dickson (1971) and Elmes *et al.* (2001) to enable a comparison to be made between the growth patterns observed in the early stages of *O. niobe* and other polyommatae species.

Host plants recorded for other *Orachrysops* species

Data were obtained from various published sources and from fellow lepidopterists of the host plants recorded for the genus *Orachrysops*. Localities for other *Orachrysops* species were visited, the females were observed ovipositing, and specimens were taken of the host plants and sent to an expert for identification.

Ova of the *Orachrysops* species were collected and it was confirmed that the larvae survived and fed on the host plant on which they were laid. High magnification photographs were taken of the eggs and the larvae that hatched from them.

RESULTS

Field observations – larvae and pupae

The 1st and 2nd instar larvae of *O. niobe* make 0.5 mm – 1.0 mm grooves in the epidermis and palisade parenchyma of the glabrous uppersides of the leaflets of *I. erecta*. When not feeding, the larvae descend to the lowest part of the plant and rest on the stem in a head-down position, making them very difficult to find in the field.

The first 4th instar larva was discovered on 27 March 2002 at 15.50 pm on a cool, cloudy day. A vertical hole about 8 mm diameter was alongside the rootstock of this plant, from which several ants emerged. Down the hole about 20 mm deep was a 4th instar larva, which was carefully removed for measurement and found to be 18 mm long x 4 mm wide, and then replaced in the hole. A sample was taken of the ants and H. G. Robertson of the South African Iziko Museum identified them as *Camponotus baynei* Arnold. The next day the larva had pupated (dimensions 12 mm x 4 mm). A few days later the pupa could not be found, so possibly the attendant ants must have taken it deeper underground.

Most of the subsequent observations were also made later in the day and early evening, when the *C.*



Figures 2 - 6. 2. *O. niobe* larval feeding marks can be seen on the 18mm diameter rootstock of *I. erecta* host plant, where 3 larvae were found at different times. 3. Rootstock of *I. erecta* showing feeding damage inflicted by larva of *O. niobe* (original diameter of 6mm reduced to 2mm) (x20). 4. 2nd instar *O. niobe* larva (2.5 mm long). 5. 3rd instar *O. niobe* larva (7mm long) showing the head shield (Photos by D. A. Edge). 6. 4th instar *O. niobe* larva (18mm long) showing an everted tentacular organ (TO) on abdominal segment A8 (top right) (Photo by L. du Preez).

baynei attendant ants appeared to be more active. On two occasions two fully-grown 4th instar larvae were found on the same *I. erecta* rootstock. In one instance the *C. baynei* originally in attendance were supplanted by a *Pheidole* species (in large numbers), and the two larvae could no longer be found. The ant attendants were *C. baynei* in thirteen out of fifteen observations made (seven 4th instar larvae; five pupae and three pupa cases), with *Camponotus berichti* in attendance in the other two cases.

Mature rootstocks of *I. erecta* are up to 18 mm diameter (Fig. 2). The holes alongside the rootstocks of *I. erecta* appear to be excavated by the *C. baynei* ants, but these holes do not lead to ant nests. *C. baynei* was only found to be nesting above ground in decayed dead wood with holes bored out by a beetle larva, and this was usually some distance away from the *I. erecta* plants.

Captive rearing with cut host plant

The size and duration of the early stages of *O. niobe* during captive rearing on cut host plant are summarised in Table 1. The few adults that were reared were dwarfs, notwithstanding their rarity in nature (Edge, 2008).

During the 2004 and 2005 captive rearing experiments a number of new observations were made. It was confirmed that the first and second instars (and presumably the third) normally consume their shed cuticle, including the head capsule, after ecdysis.

In April 2005 experiments were conducted whereby pairs of well-fed fourth instar larvae were placed in the same container with fresh host plant. Within 24 hours in each case one of the larvae disappeared and the survivor grew in size. In one

instance the act of cannibalism was observed. Whilst the prey larva was feeding on a host plant leaf, the predator larva attacked it from behind and below, through the soft ventral parts of abdominal segments A7 and A8. After penetrating the integument with its jaws, the predator larva sucked out the prey's body contents, eventually reducing it to just a skin and head shield, which was also subsequently consumed by the predator larva.

The prey larva thrashed about during the attack trying to free itself, with the tentacular organs (TOs) being very active. The predator larva increased in size from 9 mm to 11 mm before and after this attack. The attacking behaviour appears to be calculated and instinctive.

Artificial ant nests

Regular inspections of the artificial *C. baynei* ant nests showed that the ant colonies remained healthy, with stable abundances of adult ants and brood. During the 103 days that the first nest was in place, no larvae of *O. niobe* were observed inside the nest, nor were any *O. niobe* larvae seen at all. The other nest was in the field for 15 months and although the nest remained active, no larvae were observed to come into the nest. Both host plants had large numbers of *O. niobe* eggs laid on them, and there is a high probability that *O. niobe* larvae were in the immediate vicinity of the nests.

Captive rearing with live host plant and ants

The two third instar larvae that were placed on the two host plants could not be found after the first 24 hours. When the ants were given access to the plant, they were observed crawling on the plant and on the soil under the plant in the evenings, but no larvae were detected. The larvae were also not seen in the ant nest. Three months later, the plants were isolated from the rest of the experiment and the soil around the rootstock of the plants was carefully excavated. Nothing was found around the rootstock of the more healthy plant. The stems of the other plant were badly withered and many were dead. A large hole (± 20 mm) was discovered alongside the rootstock. The plant became loose and it was lifted out of the hole. A final instar *O. niobe* larva (15 mm long x 4 mm wide) was clinging to the rootstock in a hunched position. The rootstock was badly damaged (reduced to 2 mm diameter from 6 mm).

This larva was observed for the next few months with ants remaining in attendance at all times. Since both the larva and the ants were photophobic, the

Table 1. Summary of the size and duration of the early stages of *O. niobe*, reared on cut host plant.

Stage	Size	Duration
Ovum	0.6 dia x 0.3mm high	6-7 days
1 st instar	0.8 - 1.5mm*	5 - 6 days
2 nd instar	1.5 - 3.0mm*	8 - 12 days
3 rd instar	3.0 - 7.5mm*	35 - 57 days
4 th instar	7.5 - 12.0mm*	26 - 61 days
Pupa	7.5 - 8.0mm	13 - 23 days
Adult	10 - 13mm	Up to 15 days

* For the larval instars the sizes are at the start and finish of the instar.

larva was relocated to a vertical wooden box with a red perspex translucent viewing window. The box was filled with soil and a rootstock from a live plant above was placed in a groove visible through the window. Ants from the artificial nest were given access, and were observed tending the larva and imbibing secretions from the dorsal nectary organ (DNO). The larva mostly stayed on the rootstock until it became clear that the larva was eating the rootstock, cutting out deep grooves (Fig. 3). The length of the larva was now about 18 mm. The larva eventually lost interest in the rootstock, and accompanied by ants made its way to the bottom of the box where the ants had excavated a hole. The *C. baynei* ants continued to imbibe secretions from the DNO, cover the larva with soil when exposed to light, and occasionally bodily carried the larva into deeper holes that they had excavated. At no stage was there an attempt to carry the larva into the ant nest, nor were the ants observed to feed the larva by trophallaxis or any other method. When the soil was excavated again on 5 August the larva had pupated (pupa 15 mm long), still tended by the ants. The pupa was removed in mid-October and placed under a hatching cage. A full size male butterfly (forewing length 17 mm) eclosed on 3 November 2003.

Morphology of the immature stages

The following features of the morphology of the immature stages of *O. niobe* were not reported by Edge and Pringle (1996). The 2nd instar has much shorter dorsal and ventral setae than the 1st instar, and already has active TOs (Fig. 4). The head shield of the 3rd instar larva completely covers the head (Fig. 5). The 4th instar larva frequently everts its TOs (Fig. 6).

Comparative growth characteristics of polyommata larvae

The ratios between the lengths at the finish to the lengths at the start of each larval instar are depicted in Fig. 7. Note particularly the contrast between the normally phytophagous taxa, *Lampides boeticus* (Linnaeus, 1767) and *Euchrysops barkeri* (Trimen, 1893), and the myrmecophagous species *Lepidochrysops patricia* (Trimen & Bowker, 1887), *Lepidochrysops variabilis* (Cottrell, 1965) and *Maculinea arion* (Linnaeus, 1767). The difference between *O. niobe* reared purely on leaves cut from the host plant and the same species reared on live host plant, including rootstock and with ants in attendance, is also notable, particularly in the 4th instar.

Host plants and ant associates recorded for other *Orachrysops* species

The data currently available for the host plants and known ant attendants for *Orachrysops* species are summarised in Table 2. All host plants recorded so far are in the genus *Indigofera* or the closely affiliated genus *Indigastrum* and all ant associates yet known are in the genus *Camponotus*.

DISCUSSION

Phytophagy

The life histories of several *Lepidochrysops* species have already been described:

L. patricia - by Clark and Dickson (1957).

L. variabilis - by Cottrell (1965).

L. methymna (Trimen, 1862) - by Clark and Dickson (1971).

L. trimeni (Bethune-Baker, 1923) - by Claassens (1972; 1974 and 1976).

L. ignota (Trimen & Bowker, 1887) - by Henning (1983b).

L. plebeia (Butler, 1898) - by Williams (1990).

It was generally accepted from these observations that the larvae of *Lepidochrysops* are mostly myrmecophagous.

Clark and Dickson (1971) reared *Orachrysops lacrimosa* (Bethune-Baker, 1923) as far as the start of the 3rd instar, when the larvae died. When Vári (1986) separated the genus *Orachrysops* from genus *Lepidochrysops* Hedicke on adult morphological

Figure 7. Ratios between lengths at the finish and lengths at start of larval instars for six polyommata butterflies: *O. niobe* 1 = reared on cut host plant; *O. niobe* 2 = reared on live host plant with ants; *E. barkeri* and *L. boeticus* = Clark & Dickson 1971; *L. patricia* = Clark & Dickson 1957; *L. variabilis* = Cottrell 1965; *M. arion* = Elmes *et al.* 2001.

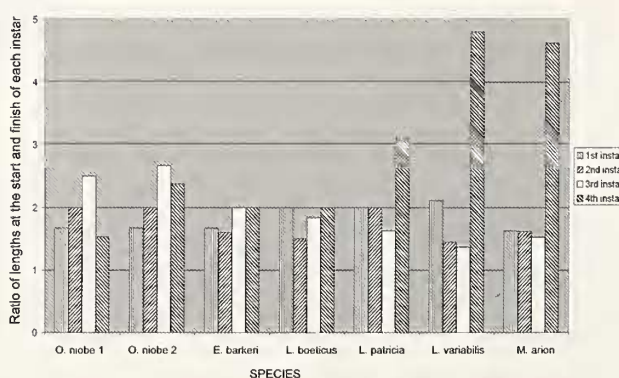


Table 2. Host plants and ant associates recorded for *Orachrysops* species.

<i>Orachrysops</i> species	<i>Indigofera</i> host plant	Locality region	<i>Camponotus</i> ant associate	Sources
<i>O. niobe</i> (Trimen)	<i>I. erecta</i> Thumb.	Brenton S. Cape	<i>C. baynei</i> Arnold	Williams, 1996; Lubke <i>et al.</i> , 1997
<i>O. ariadne</i> (Butler)	<i>I. woodii</i> var. <i>laxa</i> H. Bolus	Karloof KZN	<i>C. natalensis</i> (F. Smith)	Lu & Samways, 2001
<i>O. lacrimosa</i> (Bethune-Baker)	<i>I. obscura</i> N. E. Br.	Greylingstad Gauteng	Not known	Edge personal observations 2004
<i>O. lacrimosa</i> (Bethune-Baker)	<i>Indigastrium fastigium</i> (E. Mey.)	Verloren Valei Mpumalanga	Not known	Edge personal observations 2004
<i>O. brinkmani</i> Heath	<i>I. declinata</i> E. Mey.	Kammanassie S. Cape	Not known	Heath 1997
<i>O. subravus</i> G. A. & S. F. Henning	<i>I. woodii</i> var. <i>woodii</i> H. Bolus <i>I. tristis</i> E. Mey.	Wahroonga KZN	<i>Camponotus</i> sp.	Samways & Lu, 2007
<i>O. nasutus nasutus</i> G. A. & S. F. Henning	<i>I. elandsbergensis</i> P. B. Phillipson	Hogsback E. Cape	Not known	Lu, 2003
<i>O. mijburghi</i> G. A. & S. F. Henning	<i>I. evansiana</i> Burt Davy	Heilbron OFS	Not known	Edge personal observations 2004
<i>O. near mijburghi</i>	<i>I. dimidiata</i> Vogel ex Walp. <i>sensu stricto</i>	Heilbron OFS	Not known	Pringle <i>et al.</i> , 1994
<i>O. regalis</i> G. A. & S. F. Henning	<i>I. accepta</i> N. E. Br.	Suikerbosrand Gauteng	Not known	Terblanche & Edge, 2007
<i>O. warreni</i> G. A. & S. F. Henning	<i>I. dimidiata</i> Vogel ex Walp. <i>sensu stricto</i>	Wolkberg Limpopo	Not known	Edge personal observations 2004
<i>O. montanus</i> G. A. & S. F. Henning	<i>I. dimidiata</i> Vogel ex Walp. <i>sensu lato</i>	Verloren Valei Mpumalanga	Not known	Edge personal observations 2004
		Golden Gate OFS	Not known	Edge personal observations 2004

N. B. All *Indigofera* and *Indigastrium* plant names determined by Schrire (2005a)

grounds, the larvae of both genera were still assumed to be myrmecophagous. Edge and Pringle (1996) reared *O. niobe* larvae in captivity on host plant cuttings without ants, and this discovery added a clear biological justification for the separation of *Orachrysops* from *Lepidochrysops*.

The *O. niobe* larvae reared on host plant cuttings resulted in dwarf adults. In field observations 1st and 2nd instar larvae were nearly always found feeding on the leaves of the host plant (with a single observation of a 1st instar larva feeding on the rootstock). 3rd and 4th instar larvae were always found underground feeding on the rootstock attended by ants, and have never been found feeding on the leaves. Rootstock feeding in the Lycaenidae has only been recorded once before. Jackson (1937) observed that the larvae of *Euchrysops crawshayi crawshayi* (Butler) fed “on the fleshy outer cortex of *Cynoglossum coeruleum* Hochst. *et D.C.*, Boraginaceae, always below the ground; and they are attended by many species of ants.” Rootstock feeding in *O. niobe* appears to be essential to produce full size adults, since there is no evidence that the diet of *Orachrysops* larvae includes any ant provided

food. Rootstock feeding has also subsequently been observed in another *Orachrysops* species (Terblanche & Edge, 2007).

Rootstocks as a dietary source

Pierce (1985) noted that many myrmecophilous lycaenid larvae feed on nitrogen rich plants and nitrogen rich parts thereof (such as flowers and legume pods). More recent work has questioned this association (Fiedler, 1995; 1996), but the rootstock feeding behaviour of the 3rd and 4th instar larvae of *O. niobe* accords with Pierce (1985), if the rootstock is indeed protein rich. The rootstock of the legume *I. erecta* is a storage organ from which the plant can resprout, storing carbohydrates and bearing nitrogen fixing root nodules probably rich in amino acids and protein. Analysis of *I. erecta* leaves and rootstocks showed that although their overall amino acid content is quite similar (11600 nmol per g dry weight), the rootstocks had more than double the content of essential amino acids, particularly threonine, histidine and allo-isoleucine. These amino acids could play

an important role in the development of 4th instar larvae and pupae (e.g. threonine is important for the synthesis of collagen, a basic constituent of the more complex connective tissues generated in the lepidopteran pupal stage).

Cannibalism

Cannibalism by *O. niobe* larvae is another potent source of protein. Frequently multiple eggs are laid on a single host plant (up to 31 eggs were recorded), so encounters between 1st, 2nd and 3rd instar larvae on the host plant (where attacks are most likely to succeed) must be quite frequent. A larva grows rapidly after it has successfully attacked and consumed one of its siblings, and its survival chances are considerably enhanced. The habit that the larvae have of resting on the lower thicker part of the stem no doubt protects their vulnerable ventral parts from such attacks. Larvae that survive long enough to reach and start feeding on the rootstock are much better protected from attack, since the thick dorsal integument (carapace) extends down to the rootstock on both sides.

Morphological and behavioural adaptations of the larvae of *O. niobe*

Cottrell (1984) described a number of adaptations of lycaenid larvae that enable them to pursue a myrmecophilous life style. These adaptations have great value in not only creating “enemy-free space” (Atsatt, 1981), but also by providing access to a more reliable nutritious diet resulting in more rapid growth and larger adults (Pierce *et al.*, 2002). A number of these adaptations have been observed in the larvae of *O. niobe*. They have a thick, tough integument, which defends the larvae from attack by the ants or from conspecific larvae. They have an onisciform body shape with an expansion of the dorsal and dorsolateral areas, which slope down to well-developed seta-fringed marginal ridges that can be brought in close contact with the substrate, and seal off the ventral areas including the retracted head and legs. The ants (and the larva’s siblings) therefore cannot gain access to the softer ventral parts and vital organs when the larva crawls or rests on a hard substrate.

O. niobe larvae have a dorsal nectary organ (DNO) on the seventh abdominal segment in the 2nd, 3rd and 4th instars, producing a secretion that aids myrmecophily (Pierce, 1989; Fiedler & Maschwitz, 1989). They have tentacular organs (TOs) on the eighth abdominal segment that appear to excite the ants in attendance and draw them to the DNO (Claassens & Dickson, 1977; Fiedler & Maschwitz,

1987). The larvae of *O. niobe* also have a number of other adaptations not yet observed in other lycaenid larvae, including powerful mandibles, which adapt them for rootstock feeding and cannibalism, and an ability to burrow in the soil – although the attendant ants may assist this burrowing.

Larval shelter and overwintering

The subterranean, myrmecophilous lifestyle of the larvae of *O. niobe* insulates them from extremes of temperature and humidity changes; provides protection from fires; and gives them access to a food source (the rootstock) and a safe place to shelter during the winter when the growth of the host plants pauses.

Larval growth patterns

Dyar (1890) postulated that insect dimensions increased by the same factor at each moult. Phytocarniverous lycaenid larvae such as *Maculinea* that parasitise ant colonies are exceptions to this rule (Elmes *et al.*, 2001). *Maculinea* larvae show a normal growth pattern during the first three instars, but after adoption by their *Myrmica* ant hosts, in the final instar they increase in length by a factor of five, and by >50 times in mass (Elmes *et al.*, 1991; Thomas & Wardlaw, 1992). Elmes *et al.* (2001) hypothesised that these growth patterns may have evolved to make the newly moulted 3rd instar butterfly larvae approximately the same size as the ant larvae at adoption and better able to mimic the larvae of their ant hosts. Once in the ant nest and accepted by the ants they could grow to a larger size than other lycaenids because of the readily available, high quality food source.

In Fig. 6 it is clear that the phytocarnivorous larvae of *Maculinea* and *Lepidochrysops* show similar growth patterns. Whilst *O. niobe* larvae raised under artificial (1) or natural conditions (2) have a higher rate of growth in the third instar than any of the other examples, in the final instar the growth rate of *O. niobe* under natural conditions (2) is intermediate between the phytophagous larvae (*E. barkeri* and *L. boeticus*) and the phytocarnivorous larvae. Rootstock feeding may be the key to the higher growth achieved in the final instar relative to the normally phytophagous taxa.

Specialisation of *Orachrysops* associations

All the known host plants of the *Orachrysops* genus are in the genus *Indigofera* or the very closely affiliated genus *Indigastrium* (Table 2). Monophagy is common in the localised species and allows such species to

Table 3. Ant associations of southern African polyommata butterflies.

Key to third column [coding adopted from Fiedler (1991a)]

0 = No ant relationship (myrmecoxenous)

1 = Very few ant associations reported (weakly myrmecophilous)

2 = A varying proportion of larvae attended by ants (moderately myrmecophilous)

3 = Most if not all mature larvae ant-associated (steadily myrmecophilous)

4 = Larvae dependent on ants as commensals or parasites (obligately myrmecophilous)

** = DNO + TOs

* = DNO only

= PCOs only

() = hypothetical

? = likely but not confirmed

Genus	Known ant associates	Degree of myrmecophily	Sources
<i>Uranothauma</i> Butler		?	a
<i>Pseudonacaduba</i> Stempffer		(0/1)	c, g
<i>Cacyreus</i> Butler		(0/1)*	a, c
<i>Harpencyreus</i> Heron		(0/1)*	c
<i>Brephidium</i> Scudder		(1)**	c
<i>Ornidium</i> Bethune-Baker		(1)?	g
<i>Tuxentius</i> Larsen		(1)**	c
<i>Zintha</i> Eliot		(1)**	g
<i>Zizula</i> Chapman		(1)**	c
<i>Actizera</i> Chapman		1**	c
<i>Leptotes</i> Scudder	Indeterminate	1**	f
<i>Zizina</i> Chapman		(2)**	c
<i>Cupidopsis</i> Karsch		2**	a, c
<i>Eicochrysops</i> Bethune-Baker		2**	c
<i>Lampides</i> Hübner	<i>Camponotus</i> <i>Plagiolepis</i>	2**	c, f
<i>Zizeeria</i> Chapman	<i>Tapinoma</i>	2/3**	c, f
<i>Azanus</i> Moore	<i>Pheidole</i>	3**	a, c, f
<i>Chilades</i> Moore	<i>Pheidole</i>	3**	c, f
<i>Tarucus</i> Moore	<i>Plagiolepis</i> <i>Monomorium</i>	3**	c, f
<i>Euchrysops</i> Butler	<i>Monomorium</i>	3**	a, c
<i>Orachrysops</i> Vári	<i>Camponotus</i>	4**	i
<i>Lepidochrysops</i> Hedicke	<i>Camponotus</i>	4**	b, c, d, e

Sources: a = Jackson, 1937; b = Cottrell, 1965; c = Clark & Dickson, 1971; d = Claasens, 1974 & 1976; e = Henning, 1983a; f = Fiedler, 1991a; g = Pringle *et al.*, 1994; h = Williams, 1999; i = Lu & Samways, 2001.

avoid direct competition and co-exist at a locality (e.g. *O. lacrimosa* and *O. warreni* at Verloren Valei and *O. subravus* and *O. ariadne* at Wahroonga - Samways & Lu, 2007).

Although only two ant associations are known as yet for *Orachrysops* species, the ants concerned, *C. baynei* and *C. natalensis* have some ecological similarities (both are primarily nocturnal ants). *C. baynei* is only found in fynbos or thicket and nests in dead wood

above ground level (Edge *et al.*, 2008b), whereas *C. natalensis* can be found in fynbos as well as grassland and nests in the ground (Lu & Samways, 2002a).

Ant associates of South African polyommata

The records of known or hypothesised ant associations within the South African polyommata [members of the tribe Polyommataini as defined by

Eliot (1973)] are listed in Table 3. (Sources: Claassens, 1974, 1976; Clark & Dickson, 1971; Cottrell, 1965; Fiedler, 1991a; Henning, 1983a; Jackson, 1937; Lu & Samways, 2001; Pringle *et al.*, 1994; Williams, 1999). The coding system used in the final column has been adopted from Fiedler (1991a).

The close affinities between *Orachrysops* and *Lepidochrysops* would have always made a species of *Camponotus* the most likely host ant for *Orachrysops*, and this has now been confirmed for two of its species. However, with only 11 out of 126 *Lepidochrysops* species ant associates known (Pierce *et al.*, 2002) and 2 out of 11 *Orachrysops* ant associates known, there is insufficient evidence to conclude that *Camponotus* species are the only ant associates for both genera.

Nature of the ant association of *O. niobe*

3rd and 4th instar *O. niobe* larvae were almost always tended by the same ant, *C. baynei*. *O. niobe*'s congener *O. ariadne* has only been found in association with one ant (*C. natalensis*) (Lu & Samways, 2001). Cottrell (1984) considered that relationships specific to one ant species tended to be obligate. Even when more than one ant species interacts with the larvae, in obligate relationships one ant species is the most successful host (Thomas *et al.*, 1989). Facultative relationships by contrast tend to be formed with several species of ant, even from different genera (Fiedler, 2001).

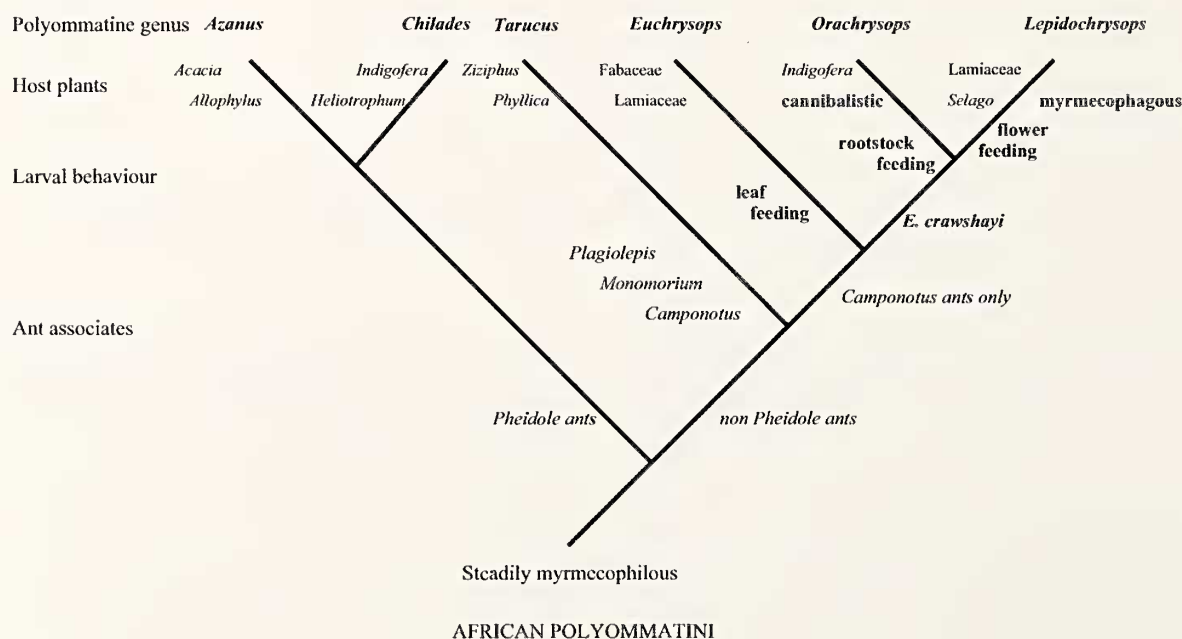
The larvae of *O. niobe* did not enter into ant nests either in the field (artificial nests) or in the laboratory. The nests of the attendant ants found to date are relatively remote from the plants on which *O. niobe* feeds, but the plants need to be within the foraging range of worker attendant ants so they can find the larvae.

No trophallaxis or other feeding of the larvae by ants has been observed. The larvae are rootstock feeders in the later instars, and appear to need the assistance of the ants to access the rootstock. After pupation, clear access to the surface through a hole or tunnel is necessary for the eclosed adult to escape and expand its wings. Ants have been observed both in captivity and in the field repositioning pupae and their assistance may be essential to place the pupa in a safe position to eclose. The balance of evidence is therefore that *O. niobe* is obligately dependent on an ant association with *C. baynei*.

Phylogenetic origins of *Orachrysops* and *Lepidochrysops*

Various authors have proposed phylogenetic hypotheses regarding the origins and evolution of lycaenid ant associations (Hinton, 1951; Eliot, 1973; Fiedler, 1991b; Pierce *et al.*, 2002). Hinton (1951) believed that the possession of a DNO was a primitive feature in the Lycaenidae, and that its absence was a

Figure 8. Hypothetical cladogram of the myrmecophilous polyommatine genera of South Africa based on genus of ant associates, larval feeding behaviour and host plant families and genera.



derived condition. Fiedler (1991b) pointed out that the lycaenid subfamilies that were apparently more primitive on other morphological considerations (Poritiinae, Miletinae and Curetinae) were not generally ant associated; whereas the more advanced subfamily Lycaeninae [sensu Eliot (1973) = Theclini + Aphnaeini + Lycaenini + Polyommagini] contained most of the myrmecophilous lineages. Within Lycaeninae sensu Eliot Pierce *et al.* (2002) predicted that the Aphnaeini and certain subtribes of Theclini would be shown to be basal and that Lycaenini and Polyommagini were derived groups.

In Table 3, southern African polyommatine genera are listed in ascending order of their degree of myrmecophily. A tentative cladogram of the steadily myrmecophilous polyommatine genera (denoted as 3 or 4 in Table 3), based on genus of ant associates, larval feeding behaviour and host plants is presented in Fig. 8. *Azanus* and *Chilades* are associated with *Pheidole* ants, which are the dominant ants in some habitats. *Tarucus* is associated with a few ant genera, including *Camponotus*. The genera *Orachrysops*, *Euchrysops* and *Lepidochrysops* are predominantly *Camponotus* associated. *Orachrysops* has evolved rootstock feeding, cannibalism, an obligate ant relationship and specialisation on *Indigofera*. *Euchrysops* has a looser ant association, and normal phytophagy, with the exception of *E. crawshayi*. This interesting taxon has genitalia similar to *Euchrysops* (Stempffer, 1967), facies similar to *Harpendyreus*, and larval behaviour with features found in *Orachrysops*. It is of note that this species has been placed in different genera by various authors (by Butler in *Scolitantides*; by Aurivillius in *Harpendyreus* and later *Cupido*; by Jackson in *Cyclirius*; and finally by Stempffer in *Euchrysops*).

The larvae of *Lepidochrysops* are phytophagous in the first two instars, and myrmecophagous in the last two instars (they feed on the ant brood). This is considered to be the closest ant relationship (Fiedler, 1998), with the larvae being treated as if they were ant brood within the ants' nests. It is here hypothesised that the larvae of the common ancestor of the three genera *Euchrysops*, *Orachrysops* and *Lepidochrysops* could have had rootstock feeding habits, which physiologically adapted them to a higher protein diet. Furthermore, the cannibalistic behaviour of *O. niobe* larvae may have evolved in the common ancestors. This would have enabled them to develop a taste and a need for insect protein, a trait that could have evolved in a sister lineage into the myrmecophagous behaviour of *Lepidochrysops* larvae.

A higher protein diet may have made closer ant associations possible because of the enhanced ability to produce nutritious (high protein) secretions from

the DNO (Pierce, 1985). This would have enabled the larvae to attract more ant attendants and given them the opportunity to evolve more advanced chemical camouflage and signaling skills (Fiedler, 1991b; 1998). Such adaptations would have generated further selective advantages in these butterfly lineages, resulting in more complex ant associations and greater interdependence of the butterflies and their ant associates.

CONCLUSIONS

O. niobe is a specialised organism, being monophagous and having an obligate relationship with a single ant species. Its habit of rootstock feeding, which may be shared with other *Orachrysops* species, distinguishes it from nearly all other afrotropical polyommatinines. A close phylogenetic relationship between the genera *Orachrysops*, *Euchrysops* and *Lepidochrysops* is inferred from a cladistic analysis.

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